Preliminarily, it is noted that the requirement for restriction set forth in the Official Action dated March 19, 2001 has been maintained and made final. Claims 40-43, therefore, have been withdrawn from consideration in the present application. Applicants hereby reserve the right to file one or more continuing applications, as the case may be, in accordance with 35 U.S.C. §120, on the subject matter of the withdrawn claims.

A number of formal objections are set forth in the September 11, 2001 Official Action, as a result of which, applicants are required to: (i) correct certain informalities noted in the drawings; (ii) add Sequence ID No. identifiers to the indicated pages of the specification; (iii) amend the specification to include a cross-reference to applicants' U.S. provisional application, the benefit of which is claimed in applicants' international application under the Patent Cooperation Treaty, on which the present National Stage application is based; (iv) submit an Abstract of the Disclosure on a separate sheet, in accordance with 37 C.F.R. §1.72(b); (v) replace the existing title of the invention with a new more descriptive title; and (vi) insert the heading "Brief Description of the Drawings" at the appropriate place in the specification. It was further noted with respect to the drawings that Figures 33 and 34 are briefly described, but that no such figures appear in the application as filed, and that Figures 27 and 30 are unlabeled.

In addition, claims 3 and 24 have been objected to as failing to further limit the subject matter of a previous claim. It is also pointed out that claim 32 should be dependent from claim 31, rather than claim 11.

Turning to the substantive aspects of the September 11 Official Action, claims 24, 27-34 and 38 stand rejected under 35 U.S.C. §112, second paragraph, as allegedly indefinite. These claims are viewed as objectionable based on the following recitations or purported implications of

recitations: the KS portion of the loading module cannot be simultaneously heterologous and homologous (claim 24); "an avr loading module" (claim 27); "nucleic acid" (claims 29 and 31-34); "leading to" and "natural unit" (claim 30); "natural activator gene" and "accompanied by" (claims 32 and 34); and "'single donor' DNA" and "heterologous chromosomal PKS DNA" (claim 28). Furthermore, claims 24, 28, 30 and 38 have been characterized as either wholly confusing or wholly unclear.

Claims 29, 37 and 39 have been further rejected for allegedly failing to comply with the written description requirement of 35 U.S.C. §112, first paragraph. It is the Examiner's position in this regard, that claim 29 requires a nucleic acid sequence encoding a chain-terminating enzyme other than thioesterase; however, according the Examiner, no such nucleic acid sequence is described, except in functional terms. As for claims 37 and 39, the Examiner notes that these claims are drawn to transformed organisms and methods of using said organisms. The Examiner acknowledges that the specification adequately describes transformed microorganisms, such as *streptomyces* species using hybrid modular PKS genes, but contends that from the genus of microorganisms disclosed, one of skill in the art could not predict the structure and/or function of the genus claimed due to the unpredictability of prokaryotic vs. eukaryotic organisms.

Claims 29 and 39 have been further rejected under 35 U.S.C. §112, first paragraph, as allegedly failing to provide a sufficiently enabling disclosure. It is the Examiner's position in this regard that undue experimentation would be required to practice the invention claimed in claims 29 and 39. Regarding claim 29, the Examiner asserts that applicants have presented no guidance or working examples for the identification of a hybrid PKS gene comprising a nucleic acid sequence encoding a chain terminating enzyme other than a thioesterase. As for claim 39, the Examiner asserts that all examples of the claimed genus described in the present

specification relate to the use of microorganisms which natively produce polyketides and that applicants present no guidance or direction for the purpose of using other organisms.

Claims 1, 31-37 and 39 have been rejected as allegedly anticipated by U.S. Patent No. 5,962,290 to Khosla et al. (hereinafter the "Khosla patent"), which is cited as prior art under 35 U.S.C. §102(e).

Claims 2, 3, 25 and 26 are allegedly unpatentable under 35 U.S.C. §103 in view of the Khosla patent, and claim 27 is likewise rejected under 35 U.S.C. §103 as allegedly unpatentable in view of the combined disclosures of the Khosla patent and Kao et al., Science 265: 509-12 (1994) (hereinafter the "Kao reference").

The above-noted objections and rejections constitute all of the grounds set forth in the September 11, 2001 Official Action for refusing the present application.

In accordance with the present amendments, the specification has been amended to include the following: the insertion of Sequence ID No. identifiers at the pages of the specification where needed; an appropriate Cross-Reference to Applicants' earlier filed U.S. Provisional application; an Abstract of the Disclosure on a separate sheet; the substitute title of the invention suggested by the Examiner; and a section heading for the Brief Description of the Drawings section of the specification. The brief descriptions of Figures 33 and 34 have been deleted from the specification, along with the specific reference to Figure 33 at page 119 of the original specification, in view of the absence of corresponding drawing figures.

Before addressing the various claim amendments, it may be helpful to provide an explanation regarding the significance of the term "loading module", which appears in original claims 2 and 3.

The concept of loading modules was introduced by the present inventors, as is explained at page 6, middle paragraph of the present specification. It was not in general use as of the filing date of the present application. Thus, the Examiner's reference to an "art-recognized definition" must refer to the situation after the filing of the present application. Previously the art-recognized concept was of a first module, which applicants regard as two modules - the loading module and the first extension module. C.f. WO93/13663 discussed in the present application at page 6 lines 23-27.

The language of claim 2 takes into account applicants' new recognition of the existence of loading modules. These modules can be used in the manner referred to in claim 2, irrespective of whether they comprise AT and ACP as specified by claim 3. In fact even at the filing date of the present application, there were published sequence data which can be interpreted as showing loading modules which differ from AT-ACP. Thus, the rapamycin loading module comprises adenylation, enoyl reductase and ACP domains (Schwecke et al., Proc. Natl. Acad. Sci., USA, 1995, 92, 7839), referred to in the present specification at page 3. The tylosin and spiramycin clusters were also shown to commence with a different domain organization, designated KS^0-AT-ACP, in which the KS^0 domain was believed to be a non-functional domain resembling KS domains (Kuhstoss et al., Gene, 1996, 183, 231). It was later shown (Bisang et al., Nature, 1999, 401, 502-505) that KS^0 was not non-functional, but acted as a decarboxylase for malonyl CoA priming units, and as a result was redesignated KS^Q.

Directing attention to the claim amendments, claim 24 has been amended to recite that at least one first nucleic acid portion encodes a loading module together with only the ketosynthase ("KS") domain of the extender module which is homologous to said loading module. As a result of this

amendment, it is respectfully submitted that claim 24 properly further limits claim 2, from which it depends.

Claim 32 has been amended so as to be dependent from claim 31. The Examiner's action in examining claim 32 as if such an amendment had been made is acknowledged with appreciation.

Claim 27 has been amended to recite that the loading module is the loading module of the avermectin-producing PKS of *Streptomyces avermitilis*.

Applicants question the propriety of the requirement that claims 29 and 31-34 be amended to recite "a nucleic acid sequence". It is noted in this connection that the well known terms "DNA" and "RNA" are abbreviations in which "NA" stands for "nucleic acid" and not "nucleic acid sequence". Moreover, these terms, as universally used, do not refer to single nucleotides. It would seem, therefore, that "nucleic acid" is an appropriate generic term which may be used to refer to DNA and RNA. Nevertheless, claims 29 and 31-34 have been amended to recite "nucleic acid sequence", in order to advance the prosecution of the present application.

Claim 30 has been canceled, thus rendering moot its rejection based on alleged indefiniteness.

Claim 32 has been further amended to recite that the nucleic acid sequence according to claim 31 further comprises the natural activator gene for said promoter.

Claim 38 has also been canceled, thus rendering moot its rejection based on alleged indefiniteness.

Also in accordance with the present amendments, new claims 44-58 are presented for consideration by the Examiner. New claim 44 corresponds generally to original claim 2, with the further recitation that the first nucleic acid portion encodes at least a loading module which comprises acyltransferase and an acyl carrier protein.

New claim 45 is an independent claim which is believed to provide a more definitive characterization of the "combinatorial module" aspect of the present invention.

New claim 46 corresponds generally to the subject matter of claim 30, but eliminates the specific language in claim 30 which the Examiner considers objectionable.

New claims 47-53 are based mainly on the disclosure at page 9, lines 32-36 and at page 16, lines 20-37 of the present specification. These claims cover the use of plasmids in greater detail than was the case with original claim 38. The plasmid may integrate or remain autonomous (see page 9, lines 32-34). A plasmid may carry the hybrid gene, or it may contain donor DNA that becomes joined to acceptor DNA in a host's chromosome, thereby creating the hybrid gene (see page 9, lines 29-33).

New claim 54 is drawn specifically to the use of the rap enzyme to replace the normal chain-terminating enzyme of PKS (usually thioesterase), thereby leading to a different type of product, as disclosed in the present specification at page 7, line 29 to the end.

Claims 55-58 are directed to transformed prokaryotic organisms which express a polyketide synthase encoded by the gene of claim 1 and to methods of making such microorganisms, which find support in the present in the present specification at page 16, lines 11-28. Insertion of donor plasmid PKS DNA into an acceptor PKS bacterial chromosome is described on page 16, lines 29-33. One of skill in the art appreciates that the term "procaryotes" and "bacteria" are often used interchangeably. Claim 56 requires that the host naturally expresses a PKS. This requirement is supported at page 18, lines 3-9 of the specification wherein it is stated that the recombinant plasmids may be introduced into bacterial cells which endogenously express PKS under the control of the actI promoter.

No new matter has been introduced into this application by reason of any of the amendments presented herewith. Moreover, none of the present claim amendments are believed to constitute a surrender of any originally claimed subject matter in order to establish patentability. Rather, the present claim amendments do nothing more than make express that which had been implicit in the claim language as originally worded.

In view of the present specification amendments, it is believed that all of the formal requirements set forth in the September 11, 2001 Official Action have been satisfied, with the exception of the requirement regarding drawing corrections. It is respectfully requested that the drawing correction requirement be held in abeyance, pending the indication of allowable subject matter.

As a result of the foregoing claim amendments, any indefiniteness or lack of clarity that may have been engendered by the previous wording of claims 24, 27, 29 and 31-34 has now been eliminated. Moreover, when the significance of "loading modules" is correctly understood, in accordance with the foregoing explanation, it is clear that claims 3 and 24 serve to further limit the claim from which they depend. Thus, the only rejections remaining to be addressed are the 35 U.S.C. §112, second paragraph, rejection of claim 28, the 35 U.S.C. §112, first paragraph rejection of claims 29 and 39 based on alleged insufficient enablement, the 35 U.S.C. §112, first paragraph rejection of claims 29, 37 and 39 based on alleged lack of written description and the various prior art rejections based on the Khosla patent alone and in combination with the Kao reference. These last-mentioned grounds of rejection are respectfully traversed for the reasons set forth hereinbelow.

A. Claim 28 Complies with the Definiteness Requirement of 35 U.S.C. §112, Second Paragraph

The relevant inquiry in determining compliance with the definiteness requirement of 35 U.S.C. §112, second paragraph, is whether the claim in question sets out and circumscribes a particular area with a sufficient degree of precision and particularity, such that the metes and bounds of the claimed invention are reasonably clear. In re Moore, 169 U.S.P.Q. 236 (C.C.P.A. 1971).

The definiteness of claim language may not be analyzed in the abstract, but must be considered in light of the supporting specification, with the language in question being accorded the broadest reasonable interpretation consistent with its ordinary usage in the art. In re Morris, 44 U.S.P.Q.2d 1023, 1027 (Fed. Cir. 1997). See also Ex parte Cole, 223 U.S.P.Q. 94 (Bd. Apps. 1983) (claims are addressed to the person of average skill in a particular art; compliance with §112 must be adjudged from that perspective, not in a vacuum).

Furthermore, it has long been held that the initial burden of establishing a failure to comply with 35 U.S.C. §112, second paragraph, rests upon the Examiner. In rejecting a claim for alleged indefiniteness, therefore, it is incumbent upon the Examiner to establish that one having ordinary skill in the art would not have been able to ascertain the scope of protection defined by the claim when read in light of the supporting specification. Ex parte Cordova, 10 U.S.P.Q.2d 1949, 1952 (PTO B.P.A.I 1988).

When the appropriate procedural approach is followed in assessing the claim terminology at issue herein, in accordance with the above-noted authorities, it is beyond question that applicants have satisfied the definiteness requirement of §112, second paragraph, with respect to the subject matter of claim 28.

The term 'combinatorial module' is explained in the present specification at page 9, lines 20-29. The nature of (extension) modules was reasonably well established by the filing date of the present application. Figs. 2a and 3 show many examples, all of the form KS.AT.*.ACP, where * represents a bond or one or more reductive domains which may be present. It might be expected that the best way to produce altered PKSs would be by replacing (at the DNA level) one natural extension module with another one, e.g. differing in the nature of *. However the present inventors found that it can be advantageous to replace a module-sized piece of DNA which consists of the end part of one module with the front part of the following module, e.g. *.ACP.KS.AT. The replacement would also be of the form *.ACP.KS.AT. It might differ from the original 'combinatorial module' in the nature of the reductive loop* (or the presence or absence thereof) and/or in the nature of the AT (e.g. to alter the specificity so that the resulting PKS might incorporate different (substituted) malonate building blocks; and/or in the nature of KS. As explained at page 2, lines 5-16 and page 9, lines 27-29, a portion to be replaced, and/or the portion to be used as its replacement, can in fact extend from a site (in this example, the AT-ACP junction) to a corresponding site not of the adjacent module but of a further module, so that the portion consists of a piece of DNA equivalent to two or more modules, e.g. of the form .ACP.KS.AT*.ACP.KS.AT. The term 'combinatorial module' is explained in some detail at page 2, lines 5-16 of the present specification.

In summary, applicants' position with respect to the rejection of claim 28 based on 35 U.S.C. §112, second paragraph, is that any person skilled in the art, having applicant's disclosure and claims before him or her, would be apprised to a reasonable degree of certainty as to the exact subject matter encompassed within claim 28. Nothing more is required under 35 U.S.C. §112, second paragraph.

For all of the foregoing reasons, it is clear that in the present case, the Examiner has failed to sustain the United States Patent and Trademark Office's burden of proof with respect to the §112, second paragraph, rejection of claim 28, as set forth in the September 11, 2001 Official Action. Accordingly, this ground of rejection is improper and should be withdrawn.

B. The Present Specification Satisfies the Written Description Requirement of 35 U.S.C. §112, First Paragraph, with Respect to the Subject Matter of Claims 29, 37 and 39

The relevant inquiry in determining compliance with the written description requirement of 35 U.S.C. §112, first paragraph, is whether the originally filed specification reasonably conveys to a person having ordinary skill in the art that, as of the application filing date, applicants had possession of the claimed subject matter. In re Kaslow, 217 U.S.P.Q. 1089 (Fed. Cir. 1983).

Furthermore, the Examiner has the initial burden of presenting evidence or reasons why persons skilled in the art would not recognize in applicants' specification disclosure a description of the invention defined by the claims. Ex parte Sorenson, 3 U.S.P.Q.2d 1462 (Bd. Pat. App. 1987).

The Examiner's argument that claim 29 lacks an adequate written description is invalid, as it is based on the demonstratably false premise that the "specification does not disclose any representative species of any of the recited classes of possible nucleic acid sequences, with or without identifying characteristics such as structure" (emphasis in original). On the contrary, the present specification specifically refers, at page 3, lines 9-16, to the rapamycin PKS and the fact that its complete sequence had been published by Schwecke et al. Moreover, at page 7 of the present specification, line 29 to the end, there is reference to

replacing "the normal chain terminating enzyme of a PKS (usually thioesterase)...by an enzyme leading to a different type of product, and specific disclosure that "use may be made of the enzyme from the rapamycin system that connects the polyketide chain to an amino acid chain".

Applicants contend that the subject matter of Claims 37 and 39 as filed is adequately described in the present specification. However, in order to advance prosecution, claims 37 and 39 have been amended to recite transformed microorganisms and methods of making polyketides using the same, in keeping with the Examiner's helpful suggestion. In light of this amendment, Applicants respectfully submit that any perceived lack of description of these claims has been obviated.

For all of the foregoing reasons, it is clear that in the present case, the Examiner has failed to satisfy the United States Patent and Trademark Office's burden of proof with respect to the lack of written description requirement rejection, as applied to the subject matter of amended claims 29, 37 and 39. Accordingly, this ground of rejection is untenable and should be withdrawn.

C. The Present Specification is Enabling with Respect to the Subject Matter of Claims 29 and 39

A rejection under 35 U.S.C. §112, first paragraph, based on inadequate enablement is proper only when the rejected claims are of such breadth as to read on subject matter as to which the specification is not enabling. In re Brokowski, 164 U.S.P.Q. 642 (C.C.P.A. 1970).

Furthermore, it is settled law that whenever the adequacy of enablement provided by the applicants' specification is challenged, the United States Patent and Trademark Office (PTO) has the initial burden of giving reasons, supported by the record as a whole, why the specification is not considered enabling. In re Armbruster,

185 U.S.P.Q. 152 (C.C.P.A. 1975). A properly supported showing that the disclosure entails undue experimentation is part of the PTO's initial burden under §112, first paragraph. In re Angstadt, 190 U.S.P.Q. 214 (C.C.P.A. 1976). Indeed, the court in Brokowski specifically stated that 35 U.S.C. §112 does not permit the Patent Examiner to study applicants' disclosure, formulate a conclusion as to what the Examiner regards as the broadest invention supported by the disclosure and then determine whether the claims are broader than the Examiner's conception of what "the invention" is. Id. at 645.

1. Enablement of Claim 29

As noted hereinabove, regarding the impropriety of the lack of written description rejection of claim 29, the present specification expressly refers to the rapamycin PKS and the fact that its complete sequence was published by Schwecke et al. See page 3, lines 9-16 of the present specification. It is well established that the patent applicants' specification need not teach, and preferably omits, that which is known in the art. Hybritech Inc. v. Monoclonal Antibodies, Inc., 231 U.S.P.Q. 83 (Fed. Cir. 1986), and authority cited therein. Furthermore, the present specification at page 7, line 29 to the end, refers to replacing "the normal chain terminating enzyme of a PKS (usually thioesterase)...by an enzyme leading to a different type of product", and specifically discloses that "use may be made of the enzyme from the rapamycin system that connects the polyketide chain to an amino acid chain".

Thus, the conclusion of the Examiner in paragraph 24 that "no other enzyme catalyzing such an activity is identified in the art...or in the specification" is factually incorrect.

2. Enablement of Claim 39

The Examiner refers to remarks in a paper by Kao et al. that expression of recombinant PKSs can be difficult. With all due respect, it is respectfully submitted that Kao's remarks have limited validity, and/or the Examiner is misinterpreting their significance. For example, there is substantial illustration in the prior art of the proper folding and assembly of intact PKS proteins in *E. coli* (a heterologous host which does not natively produce any polyketide) - see Roberts *et al (Eur. J. Biochem.*, 1993, **214**, 305-311) and Leadlay *et al, Biochem Soc Transactions*, 1993, **21**, (1), 218-222. Furthermore, any problems of manipulation of large pieces of DNA could apply only if large pieces need to be moved, and would not apply, for example, to small PKSs (e.g. those producing triketide lactones) or where only a smaller fragment of a PKS were required to be transformed into a host. Moreover, Kao *et al* show that manipulation of an entire set of PKS genes is technically possible. Techniques, including those of Kao *et al*, were therefore available in the prior art. The issue of substrate supply does not preclude transformation of the organism and expression of the polyketide synthase (which is the substance of claims 37 and 39). Both traditional microbiological (mutagenesis, fermentation development) and recombinant methods of influencing substrate supply are well known in the art. With respect to recombinant approaches, for example, McKie *et al, Biochem. J.*, 1990, **269**, 293-298, describes the heterologous expression, as the active holoenzyme, of the methylmalonyl CoA mutase (involved in methylmalonlyl CoA supply) from *Propionibacterium shermanii* in *E. coli*, and Wallace *et al, Eur. J. Biochem.*, 1995, **233**, 954-962 describes the purification of the crotonyl CoA reductase (ccr) gene (involved in butyryl CoA supply) and its expression in *E. coli*. Use of the ccr gene to augment butyryl CoA, and

therefore ethylmalonyl CoA, supply is exemplified in Examples 53-56 (see page 102), to produce ethyl substituted triketide lactones and erythromycin analogues.

On the present record, it is clear that the PTO has failed to present evidence sustaining its burden of proof under §112, first paragraph, insofar as enablement for claims 29 and 39 is concerned. At the very least, the Examiner should substantiate by evidence or sound reasoning that the claims read on subject matter as to which the present specification is considered non-enabling.

In view of the absence of evidence or reasoning tending to substantiate the doubts expressed by the Examiner regarding the scope of enablement provided by the present specification, the rejection of claims 29 and 39 based on allegedly inadequate enablement is clearly improper and should be withdrawn.

D. The Disclosure of the Cited Khosla Patent Fails to Anticipate Claims 1, 31-37 and 39

The Examiner places considerable emphasis on the claims of the Khosla patent in stating this ground of rejection. No fewer than six (6) claims of the Khosla patent are relied on in support of the Examiner's position. As was observed, however, in In re Benno, 226 U.S.P.Q. 683 (Fed. Cir. 1985), at 686-87:

> The scope of a patent's claims determines what infringes the patent; it is no measure of what it discloses. A patent discloses only that which it describes, whether specifically or in general terms, so as to convey intelligence to one capable of understanding. While it is true...that 'a claim is part of the disclosure,' that point is of significance principally in the situation where a patent application as filed contains a claim which specifically discloses something not disclosed in the descriptive part of the specification (claims being

> technically part of the 'specification,' (35 U.S.C. §112, 2d par.), in which case the applicant may amend the specification without being charged with adding a 'new matter,' within the meaning of §132...But that is not the situation here. [The claim of the prior art patent] does not disclose any structure additional to what the [prior art patent's] specification discloses.

Accordingly, it is necessary to determine precisely what the cited Khosla patent "describes, whether specifically or in general terms, so as to convey intelligence to one capable of understanding". For this purpose, it is worthwhile to review the file history of the cited Khosla patent.

The Khosla patent was filed as U.S. Application No. 08/949,943 on October 14, 1997, i.e. after applicants' priority date of July 5, 1996 and PCT filing date of July 4, 1997. However, it is designated a divisional of U.S. Application No. 08/486,645, issued as U.S. Patent No. 5,712,146, filed June 7, 1995.

From the file wrapper of the Khosla patent, enclosed are copies of:

1. Index of claims (Exhibit A);
2. Claims as originally filed (claims 1-50 on sheets 98-105) (Exhibit B);
3. Copy of Second Preliminary Amendment filed June 3, 1998 (Exhibit C);
4. Copy of Third Preliminary Amendment filed October 26, 1998 (Exhibit D);
5. Copy of Fourth Preliminary Amendment filed October 28, 1998 (Exhibit E); and
6. Copy of pages 8, 18, 19 and 29 of the application as filed (Exhibit F).

In applying the Khosla patent in this case, the Examiner principally relies on certain claims in the granted patent, i.e. claim 10 especially, and also claims 11, 12, 17, 18 and

19. As shown by the Index of Claims, these correspond to original claims as follows:

Claim No. in Patent	Original Claim No.
10	60
11	61
12	62
17	77
18	78
19	79

All of the above-listed claims were added by the Second Preliminary Amendment (Exhibit C). Claims 10 (60), 17 (77) and 19 (79), were further amended by the Third Preliminary Amendment (Exhibit D). No similar claims were present in the application as filed. These claims (or the first versions of them) were not filed until June 1998. Not only is this long after the filing date of the present application, it is about five (5) months <u>after</u> publication of applicants' corresponding PCT application on January 15, 1998. Thus, these claims represent a reaction to applicants' invention, and not an anticipation of it.

The description in the Khosla patent is <u>almost</u> the same as in the application as filed, which appears to be the same as the description in the parent patent U.S. Patent No. 5,712,146. However it includes some late amendments.

The Third Preliminary Amendment (Exhibit D) altered a passage at page 29 of the application text, affecting the first full paragraph in column 15 of the patent.

The Fourth Preliminary Amendment (Exhibit E) affected the brief description of Fig. 22 in column 9 (page 18 of the applicant text).

The additions and alterations made after the present applicants's filing and publication dates added new concepts to the application that were not originally present.

Furthermore they are liable to influence the interpretation of other, unaltered passages in the Khosla patent. These must of course be read in their original context (as filed in June 1995) and not in the 1998-influenced context provided by the Second, Third and Fourth Preliminary Amendments.

1. What is the "Prior Disclosure" in Khosla?

(a) Claim 10

The Examiner states in paragraph 27 of the September 11, 2001 Official Action that Khosla et al. teach a "DNA molecule which comprises a recombinant expression system for production of a hybrid modular (Type 1) PKS...wherein said activities [KS, AT, ACP etc.] are derived from at least two different modular PKS" (see claim 10)." The wording quoted by the Examiner is present in claim 10, but not in any part of Khosla which has the status of prior art. As noted above, claim 10 is derived from claim 60, filed in the Second Preliminary Amendment. It was there stated that new claims 60-67 were supported by page 19 lines 17-22 (sic lines 16-22) and page 29, first full paragraph.

Page 19 lines 16-22 of the Khosla et al. application corresponds to column 10 lines 23-29 of the patent. This is in a section headed "Definitions", in a passage defining the term "replacement PKS gene cluster". It is merely setting out how the term will be used, and not disclosing the invention. The most pertinent passage is:

> Hybrid clusters can include genes derived from both type I and type II PKSs" (Khosla patent column 10 lines 27-29).

This is merely a statement about how the term "hybrid cluster" may be used. It implies that the term encompasses a cluster containing genes of both type I and type II. It does not, even implicitly, disclose a hybrid gene cluster in which both elements are type I. It is in any case a mere definition. It is certainly not an enabling disclosure. It is not even a statement about what Khosla et

al. regard as their invention. Its function is merely to define a term that appears in the specification.

Page 29, first full paragraph of the Khosla et al. application, provides a list of gene clusters without an indication as to their classes. The list is preceded by the wording:

> Examples of hybrid replacement clusters include clusters with genes derived from two or more of.

This clearly fails to constitute a disclosure of the concept of type I/type I hybrids, as called for in applicants' claims. Indeed in the context of the Khosla et al. application, it is much more suggestive of type II/type II hybrids. Thus the preceding paragraph (column 14 line 61 of the printed Khosla patent) refers to "a gene for one cluster replaced by the corresponding gene from another gene cluster". In a type II system, a PKS has a single active site (enzyme activity) that is used in every cycle of chain extension. Thus it is meaningful to talk about "corresponding" active sites, or genes therefor, of different systems. In contrast a Type I PKS has a different set of active sites for each cycle of extension. Thus to refer to a "corresponding gene from another gene cluster" would have no apparent meaning in this context.

(b) Column 14 lines 26-35

In section 27 the Examiner further states:

> Khosla et al teach examples of genes for use in hybrid nodular (sic) PKS clusters such as erythromycin...and candicidin (see column 14, lines 26-35).

The Examiner's position in this regard is not altogether clear. A passage similar to that referred to by the Examiner appears in column 15 of the cited Khosla patent ("Khosla '290"). The Examiner was apparently looking at the related passage in Khosla 5,712,146 (the parent case, "Khosla

'146"). In fact it is more appropriate to look at the passage in Khosla '146 since this corresponds exactly to the passage as originally filed in Khosla '290, on page 29 of the application text. As explained above, this was amended by the third preliminary amendment to produce the passage now in column 15 of Khosla '290. The original paragraph is fully discussed in section (a) above.

(c) Claim 17

In the September 11 Official Action, the Examiner asserts that:

> Khosla et al. further teach said DNA molecule operably linked to an actinorhodin (act) promoter (see claim 17).

As explained above, claim 17 of Khosla '290 is not prior art. It was stated in the Second Preliminary Amendment that claim 77 (which became claim 17) was supported by Example 7. Example 7 is unusual in Khosla '290 in that it clearly does refer to a type I PKS gene cluster. However, it has no teaching at all about any form of hybrid. It discloses that "a shuttle plasmid containing the complete *ery* A genes, which were originally cloned from pS1...was constructed" (column 42 lines 37-38). Ultimately, *S. coelicolor* is transformed, and expresses 6dEB.

Example 7 clearly depends on homologous recombination to integrate some *ery* genes on the conditionally-replicating plasmid at a restrictive temperature into the *ery* cluster. There is nothing novel in this example and practitioners in the field have been using such homologous recombination to integrate DNA into actinomycetes for years. Perhaps the first demonstration was 'mutational cloning' (Chater KF, Bruton CJ. (1983) Mutational cloning in *Streptomyces* and the isolation of antibiotic production genes. Gene. **26:**67-78.). The use of conditional-temperature plasmids

in an actinomycete context was described in a number of publications by Gunter Muth in the late 1980's.

There is no description in claim 17 or Example 7 of Khosla '290 of a DNA molecule which is a hybrid type I/type I gene cluster operably linked to anything.

(d) Column 19 lines 38-40 (see page 15 line 15 of the September 11, 2001 Official Action)

Once again the Examiner appears to be referring to Khosla '146. The passage is also in Khosla '290, column 20 lines 26-29. The whole paragraph, which refers to example 7, concerns the problems of cloning *ery* A genes and has nothing to do with hybrids.

(e) Claims 11 and 18

These claims are cited by the Examiner at page 15, line 16, as teaching "host cells containing said DNAs". As already explained, claims 11 and 18 are not prior art. They correspond to earlier claims 61 and 78. The alleged basis for claim 61 is the same as for claim 60 (now 10) and is discussed in section (a) above. Again, there is no teaching in Khosla '290 suggestive of type I/type I hybrids. The alleged basis for claim 78 is example 7, which is discussed in section (c) above. It does not disclose hybrids at all.

(f) Claims 12 and 19

These claims are cited at page 15, line 18 of the September 11 Official Action. They derive from claims 62 and 79. They are not prior art. The alleged bases are the same as those referred to in section (e), and they are no more relevant here.

(g) Column 3 lines 7-10

This passage is cited at the foot of page 15 of the September 11 Official Action. Once again the Examiner appears to be referring to Khosla '146, but the same wording is present in column 3 of Khosla '290 in lines 11-13. It is an entirely general statement that does nothing to supply the absence of specific disclosure of type I/type I hybrids.

(h) Column 4 lines 44-65; Column 9 lines 38-50; Column 13 lines 53-58; Column 25 lines 24-40

These passages are referred to at page 16, line 2 of the September 11 Official Action. It is not clear which prior art document the Examiner is referring to. In both Khosla '290 and '146, column 4 lines 44-65 constitutes a part of a statement of an embodiment of the invention which begins before line 44, and runs over into column 5. The other passages appear to be cited from Khosla '146 so that reference will be addressed in the following discussion.

The statement bridging columns 4 and 5 refers to a method in which one or more first modules and an assortment of second modules (clearly it is intended to refer to DNA encoding modules) undergo homologous recombination to produce "recombinant PKS gene cluster modules". This does not amount to a clear disclosure of a hybrid encoding at least one domain which is heterologous to the other PKS. It is noteworthy that this 'statement' does not correspond to anything which is exemplified in either Khosla '146 or '290. Indeed the present inventors are not aware that such a method has ever been carried out. It would require recombination *in vivo* of non-identical DNA sequences. A search of the literature relating to actinomycetes has failed to find a single example of such recombination. Indeed later publications of Khosla et al. show that they themselves do not consider that type I/type I hybrids were made available by the disclosures of their '146 and '290 patents.

Statements of Khosla et al. from publications dating from after Khosla '146 and '290 provide some guidance as to how much genuine disclosure they contain.

WO98/49315 (filed April 30, 1998 claiming priority of April 30, 1997 and March 5, 1998) relates to type I PKSs. In a still later application of Khosla et al. (WO 01/27284) it is stated: "This system also expedited

construction of the first combinatorial modular polyketide library in *Streptomyces* (see PCT Publication No. WO 98/49315)". This is a tacit acknowledgment by Khosla et al. that their earlier disclosures, including Khosla '146 and '290, do not disclose combinatorial (i.e. hybrid) modular (i.e. type I) PKS systems.

There are numerous papers published by the Khosla group after the application for Khosla '290 was filed showing that they clearly did not consider that a process of recombining modules (as Khosla '290, column 4 lines 43 - column 5 line 6 purports to suggest) was actually known. For example, Gokhale et al, 1999, Science, 284, 482-485 states at page 482-3 para 2: 'An alternative strategy for combinatorial biosynthesis would be to recombine intact modules from the vast natural repertoire of PKSs. Such an approach would benefit from the use of highly evolved modules as intact catalytic units, thereby eliminating unwanted perturbations in module structure or chemistry..... However, productive chain transfer between heterologous PKS modules has not been reported, perhaps because of the lack of understanding of the molecular basis for intermodular communication.'

Further on page 485, the concluding sentence reads: 'Second, it provides a fundamentally new strategy for combinatorial biosynthesis, in which modules rather than individual enzymatic domains, are the building blocks for genetic manipulation.'

Tsuji et al, Biochemistry, 2001, 40, 2326-2331 state at page 2326 para 1: 'Since PKS modules comprise natural, integrated catalytic units, reorganising entire modules, rather than individual domains, provides an attractive method for exploiting the combinatorial potential of this biochemistry. Before realising this strategy, the recognition features of modules need to be defined.'

Such references show that, even in 2001, the process purportedly referred to in Khosla '290 was still not yet achieved by Khosla and his co-workers.

Column 9 lines 38-50 of 146 corresponds to column 10 lines 23-44 of Khosla '290. The main part of this is already discussed in (a) above.

Column 13 lines 53-58 of Khosla '146 corresponds to column 14 lines 30-35 of Khosla '290. It describes nothing suggestive of type I/type I hybrids.

Column 25 lines 24-40 of Khosla '146 corresponds to column 26 line 56 - column 27 line 5 of Khosla '290. This passage means no more than "Type 1 PKSs seem likely to be an interesting field of study". The lack of any specific information here is indicative of the low level of disclosure relating to type I systems in the two applications for Khosla '146 and '290. The few references in these applications that do clearly relate to type I systems merely repeat material that was already in the prior art.

(i) Column 42 lines 39-41

This passage is cited on page 16 of the September 11 Official Action, 5 lines from the bottom and appears to be an intended reference to Khosla '146 column 42 lines 39-42 (present in Khosla '290 at column 43 lines 15-18). This is part of Example 7, discussed in section (c) above. The *ery* A genes are expressed in *S. coelicolor*. In addition to the expected product, there was a minor product which had incorporated acetate instead of propionate as the starter. This is disclosed merely in the context of this specific example (which has no teaching relevant to any types of hybrid). It does not even draw the conclusion that the DEBS loading module has a related substrate specificity, and certainly does not suggest that this should be exploited.

E. The Cited Khosla Patent Fails to Render Obvious Claims 2, 3, 25 and 26

It is well-established that all claim recitations must be considered in determining non-obviousness under 35 U.S.C. §103. In re Saether, 181 U.S.P.Q. 36 (C.C.P.A. 1974). Indeed, when the Examiner disregards specific claim recitations that distinguish over the prior art, the rejection is improper and will be overturned. In re Glass, 176 U.S.P.Q. 489 (C.C.P.A. 1973).

Applicants' claim 1 calls for a hybrid PKS gene comprising at least one first nucleic acid portion encoding at least one domain of a first type I PKS and at least one second nucleic acid portion encoding at least one I PKS domain which is heterologous to the first PKS. Such a gene is nowhere disclosed or suggested in the Khosla patent. It is incorrect to state, as the Examiner does at page 17, lines 2-3 of the September 11 Official Action that "Khosla et al. particularly teach all domain combinations for hybrid PKS gene clusters using the noted modular PKS genes." The use of the term "particularly teach" is especially inappropriate since the disclosure in Khosla relating to hybrids involving type I genes is all at an extremely vague, generic level with no specific suggestion of type I/type I hybrids, and certainly no enabling disclosure in this regard.

In view of the lack of any clear teaching in the Khosla patent of type I/type I hybrids, this reference cannot properly be relied on as evidence of unpatentability in the present case. Accordingly, the rejection of claims 2, 3, 25 and 26 based on the Khosla patent should be withdrawn.

F. The Combined Disclosures of the Khosla Patent and the Kao Reference Fail to Render Obvious Claim 27

In stating this ground of rejection, the Examiner particularly refers to column 25 lines 24-37 of the Khosla

patent. This appears to be a reference to Khosla '146, a passage discussed in section (h) above. Nowhere in either Khosla document (as originally filed) is there a specific disclosure of type I/type I hybrids. This passage does not disclose hybrids at all. Looking at the entire paragraph (Khosla '146 col. 25 lines 24-52; Khosla '290 col. 26 line 56 - col. 27 line 17), it is wholly speculative and the speculation appears to relate to the possibility of producing mixtures of polyketides by making use of (a) relaxed specificity for starter units; and (b) possible genetic manipulation. The latter is particularly speculative, in view of remarks such as "it remains to be seen to what extent this property can be manipulated" (Khosla '146, col. 25, lines 46-48). This is so far from being an enabling disclosure that it can scarcely be interpreted without hindsight. No teaching or practical suggestion of type I/type I hybrids can be gleaned from the disclosure of the Khosla patent, and additional reliance on the disclosure of Kao does not alter this conclusion.

Since the Kao reference fails to make up for the above-noted deficiencies in the disclosure of the Khosla patent, the combined disclosures of these references cannot properly constitute evidence of the obviousness of claim 27. That being the case, the rejection of claim 27 based on the Khosla patent and the Kao reference is untenable and should be withdrawn.

In view of the present amendments and the foregoing remarks, it is respectfully urged that the rejections and objections (other than the requirement for drawing corrections) set forth in the September 11, 2001 Official Action be withdrawn that this application be passed to issue, and such action is earnestly solicited.

For all of the above-stated reasons, the §102(e)

rejection of claims 1, 31-37 and 39 based on the Khosla patent cannot be maintained.

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PJH:ksk
Enclosures: Exhibits A-F

Marked-Up Copy of the Specification

At page 1 of the specification, please delete [POLYKETIDES AND THEIR SYNTHESIS] and insert-- Hybrid Polyketide Synthases Combining Heterologous Loading and Extender Modules--.

At page 19, line 37, please insert the following: Brief Description of the Drawings

At page 21, lines 36-37 over to page 22, line 2, please delete the references to Figs. 33 and 34 as follows.

[Fig. 33 shows the integration of PALVD into the genome of S. erthythraea NRRL2338; and
Fig. 34 shows the integration of PALVD into the genome of S. erthythraea TER43.]

At Page 119, lines 16-28, please replace the existing paragraph with the following to omit reference to Fig. 33.
(ii) Construction of S.erythraea ERM D1
Approximately 5-10 µg of pAVLD, isolated from E. coli TG1recO(pAVLD) was transformed into S. erythraea NRRL2338 and stable thiostrepton resistant colonies were isolated. One of these colonies was selected and total DNA was digested with PstI and analysed by Southern hybridisation employing as a probe the insert from plasmid pCRc which contains the fragment of the ery AI gene encoding the ketosynthase domain KS1. The analysis showed positively-hybridizing PstI fragments of 8.5 kbp, 4.8 kbp and 33 kbp, indicating the presence of two tandemly integrated copies of pAVLD [(Figure 33)].

Please amend the specification to insert the following SEQ ID NOS: where indicated:

(Page 26, Line 6) The 1.6 kbp DNA segment encoding the loading domain of the erythromycin polyketide synthase from nucleotide 1 to 1680 was amplified by PCR employing the CloneAmp procedure (Raschtian, A. et al. Anal. Biochem. (1992) 91: 91-97) with the following two oligonucleotides as primers: 5'-ACGCGUACUAGUCCGATTAATTAAGGAGGACCATCATGGCGGACC TGTCAAAGCTC-3' (SEQ ID NO: 1) and 5'-AUGGAGAUCUCUCCGCTAGCGGTTCGCCGGGCGCCGCTTCGTTGGTCCGC GCGCGGGTTTCCC-3' (SEQ ID NO: 2) and using as template the DNA of plasmid pNTEP2. Approximately 30-60 ng of the PCR product (1.6 kbp) is digested with uracil DNA glycosylase for 30 minutes at 37°C in the presence of 25 ng of pAMP18 vector DNA (Gibco BRL), the mixture is cooled on ice and used to transform E. coli TG1recO and individual colonies are checked for their plasmid content. The desired plasmid is identified by its restriction map and is designated pARLD

(Page 27, Line 14) pCJR101 (Figure 6) is a shuttle plasmid constructed to be used for expression of PKS genes in actinomycetes. It includes a ColEI replicon to allow it to replicate in E. coli, an SCP2* low copy number Streptomyces replicon (Bibb, M. J. and Hopwood, D. A. J. Gen. Microbiol. (1981) 126:427) and the actII-orf4 activator gene from the act cluster which activates transcription from the act promoter during the transition from growth phase to stationary phase in the vegetative mycelium. It is constructed as follows: an approximately 970 bp DNA fragment from pMF1015 (containing the actII-orf4 activator gene) (Fernandez-Moreno, M. A. et al. Cell (1991) 66:769-780) is amplified by PCR, using as primers the synthetic oligonucleotides: 5'-ACT AGT CCA CTG CCT CTC GGT AAA ATC CAG C-3' (SEQ ID NO: 3) and 5'-CTT AAG AGG GGC TCC ACC GCG TTC ACG GAC-3' (SEQ ID NO: 4), which also introduces flanking SpeI and AflII restriction sites. This fragment is introduced into the end-repaired AatII site of plasmid pUC19 to yield plasmid p18.14 (renamed pCJR18). An approximately

215 bp DNA fragment is amplified from pMV400 which contains the bidirectional promoter pair PactIII/PactI) (Parro, V. et al. Nucl. Acids Res. (1991) 19:2623-2627), using as primers the synthetic oligonucleotides 5'-ACA TTC TCT ACG CCT AAG TGT TCC CCT CCC TGC CTC-3' (SEQ ID NO: 5) and 5'-GTG ATG TAT GCT CAT ATG TGT CCT CCT TAA TTA ATC GAT GCG TTC GTC CGG TG-3' (SEQ ID NO: 6), which also introduces flanking NdeI and AflII sites. The PCR product is digested with NdeI and AflII and ligated with the plasmid p18.14 (pCJR18) previously cut with NdeI and AflII, to generate plasmid p19.4 (renamed pCJR19). A 1.1 kbp HindIII-SphI fragment containing the tsr gene, which confers resistance to thiostrepton, is obtained by PCR from plasmid pIJ922 (Lydiate, D. J. et al. Gene (1985) 35:223-235) as template, using as primers the oligonucleotides 5'-TGA ACA CCA AGC TTG CCA GAG AGC GAC GAC TTC CCC-3' (SEQ ID NO: 7) and 5'-GAC AGA TTG CAT GCC CTT CGA GGA GTG CCC GCC CGG-3' (SEQ ID NO: 8) which also introduces flanking HindIII and SphI sites. The PCR product is digested with HindIII and SphI and ligated with plasmid p19.4 (pCJR19) cut with HindIII and SphI to obtain plasmid p20.5 (pCJR24). The plasmid pIJ922 is digested with BamHI and SstI and the fragment containing a portion of the fertility locus and the origin of replication (Lydiate, D. J. et al. Gene (1985) 35:223-235) is ligated into pUC19 digested with BamHI and Sst I to generate the bifunctional plasmid p16/2.2 (renamed pCJR16) (14.7 kbp). Plasmid p20.5 (pCJR24) is digested with SalI and SphI, the two larger fragments from the digest are purified by gel electrophoresis, and combined in a four-component ligation with plasmid 16/2.2 (pCJR16) which has been digested with XhoI and SphI. The ligation mixture is used to transform Streptomyces lividans and colonies are selected in the presence of thiostrepton. One such colony is shown to contain the desired plasmid pCJR101 (approx. 12.4 kbp), identified by its restriction pattern.

(Page 28, Line 37) The construction of plasmid pCJR29 (pCJR110) is illustrated in Figure 7. A 1.1 kbp HindIII-XhoI fragment containing the tsr gene, which confers resistance to thiostrepton, is obtained by PCR from plasmid pIJ922 as template, using as primers the oligonucleotides 5'-TGA ACA CCA AGC TTG CCA GAG AGC GAC GAC TTC CCC-3' (SEQ ID NO: 7) and 5'-GAC AGA TTC TCG AGC CTT CGA GGA GTG CCC GCC CGG-3' (SEQ ID NO: 9) which also introduces flanking HindIII and XhoI sites. The PCR product is digested with HindIII and XhoI and ligated with plasmid 16/2.2 (pCJR16) which has been digested with HindIII and XhoI, to generate plasmid 22.1 (pCJR25) . Plasmid p22.1 (pCJR25) is digested with HindIII and SphI and ligated with plasmid p19.4 (pCJR19) which has been digested with HindIII and SphI, to produce the desired plasmid pCJR29 (pCJR110) (approx. 12.4 kbp), identified by its restriction pattern. Plasmid pCJR29 (pCJR110) differs from pCJR101 in the orientation of the tsr gene, the actII-orf4 gene and the actI/actIII promoter, with respect to the SCP2*-derived origin of replication.

(Page 28, Line 22) Plasmid pRM52 is a derivative of plasmid pRM5 (McDaniel, R. et al. Science, (1993) 262:1546-1550). pRM5 was first linearised by digestion with NdeI, end-repaired and then religated to produce pRM51. pRM51 was cut with PacI and NsiI and the large PacI-NsiI fragment was isolated and ligated to a short double-stranded oligonucleotide linker containing an NdeI site and constructed from the synthetic oligonucleotides 5'-TAAGGAGGACACATATGCA-3' (SEQ ID NO: 10) and 5'-TAATTCCTCCTGTGTAT-3' (SEQ ID NO: 11) which were annealed together. The ligation mixture was transformed into E. coli TGIrecO and isolated colonies were screened for their plasmid content. The desired plasmid (19.6 kbp) was identified by its restriction map and was designated pRM52.

(Page 30, Line 25) A 4.0 kb KpnI fragment extending from 1.4 kbp upstream of the correct eryAI start codon as previously determined (Caffrey, P. et al. FEBS Letters (1992) 304:225-228), to 2.6 kbp inside the eryAI gene of S. erythraea, was excised from plasmid pBK25 (Bevitt, D. J. et al. Eur. J. Biochem. (1992) 204:39-49) and cloned into pUC18 to obtain plasmid pBK6.12. DNA of this plasmid was used as the template for a PCR reaction to obtain a 360 bp product in which a unique Nde I site is created at the start codon of eryAI and a unique SmaI site is created at the other end of the PCR product. The oligonucleotides used were 5'-CCC ATA TGG CGG ACC TGT CAA AGC-3' (SEQ ID NO: 12) and 5'-ATT GCG CGC CCT GGC CCG GGA A-3' (SEQ ID NO: 13). The product was end-repaired and ligated into SmaI cut pUC18, and transformed into E. coli TG1recO.

(Page 32, Line 3) A ClaI-EcoRI polylinker, bearing unique restriction sites for XbaI and for HindIII was constructed, from the following complementary synthetic oligonucleotides:
5'-AATTCATAGTCTAGAAGCTTAT-3' (SEQ ID NO: 14)
and
5'-CGATAAGCTTCTAGACTATG-3' (SEQ ID NO: 15)
The polylinker was ligated into plasmid pNTE5, which had been digested with ClaI and EcoRI to remove a 2.3 kbp ClaI-EcoRI fragment. The ligation mixture was used to transform E. coli TG1recO and individual colonies were screened for their plasmid content. One plasmid containing the polylinker was identified and designated pNTEP2.

(Page 35, Line 6) Plasmid pCRabc (Figure 10) was constructed as follows. Three separate PCR reactions were conducted: First, 20 pmol each of synthetic oligonucleotides A1 (5'-CTC GTC GGT GGC TTT GCG-3'; SEQ ID NO: 16) and A2 (5'-CCC GGG AAA AAC GAA GAC TAG TGG CGC GGA CGG CCG-3'; SEQ ID NO: 17) were used to amplify a 1.0 kbp product from 100 ng pNCO12

template. The PCR product was end-repaired, phosphorylated and cloned into SmaI-cut pUC18 to obtain plasmid pCRa. Secondly, 20 pmol each of synthetic oligonucleotides C1 (5'-CAC GCG CAG CGC GGC GGA-3'; SEQ ID NO: 18) and C2 (5'-CGAA CCG CTA GCG GTC GTC GCG ATG GCC T-3'; SEQ ID NO: 19) were used to amplify a 1.5 kbp product from 100 ng pNC012 template. The product was end-repaired, phosphorylated and cloned into SmaI-cut pUC18 to obtain plasmid pCRc. Thirdly, 20 pmol each of synthetic oligonucleotides B1 (5'-GTGGCCCGGCCGTCCGCGCCACTAGTCTTCGTTTTT-3'; SEQ ID NO: 20) and B2 (5'-AACAGCTAGCGGTTCGTCCGCCGCTGCCGTGCC-3'; SEQ ID NO: 21) were used to amplify a 1.4 kbp product from 100 ng pVE3.4 template. The product was end-repaired, phosphorylated and cloned into SmaI-cut pUC18 to obtain plasmid pCRb.

(Page 42, Line 7) Plasmid pNEWAVETE was digested with EcoRI and HindIII and the vector was purified by gel electrophoresis. A synthetic oligonucleotide double-stranded insert encoding a 6-histidine tag and possessing these sites at either end (shown below) was ligated to the vector.

(5'-AATTCACATCACCATCACCATCACTAGTAGGAGGTCTGGCCATCTAGA-3'; SEQ ID NO: 22)
(3'-GTAGTGGTAGTGGTAGTGATCATCCTCCAGACCGGTAGATCTTCGC-5'; SEQ ID: 23)

(Page 44, Line 19) For the PCR amplification of DNA for module 1, the following synthetic oligonucleotides were used as mutagenic primers, one containing an EcoRV site and the other a BglII site:
5'-GCAGGGATATCGCACGTTCCTGG-3' (SEQ ID NO: 24)
and 5'-CGCCGAGATCTGCGAAGGCCTGGTCGGCGGG-3' (SEQ ID NO: 25)

(Page 44, Line 37) For PCR amplification of the DNA for the 5' end of module 2 and the thioesterase domain, the

following oligonucleotides containing respectively a Bgl II site and an EcoRI site, were used as mutagenic primers:

5'-ATGAATTCCCTCCGCCCAGCCAG-3' (SEQ ID NO: 26)
and
5'-ACAGATCTCGGCTTCGACTCGCTGACCG-3' (SEQ ID NO: 27)

(Page 48, Line 1) The 4.7 kbp DNA segment of the rapC gene encoding module 12 of the rapamycin PKS was amplified by PCR employing the CloneAmp procedure (Raschtian, A. et al. Anal. Biochem. (1992) 91:91-97) and with the following two oligonucleotides as primers:
5'-ACGCGUACUAGUCAGATCTGGGCATCAATTCGCTGACCGCGGTGGAACTGCGCAA-3' (SEQ ID NO: 28)
and 5'-AUGGAGAUCUCUCAGATCTTGAATGCGGCGGCTGCGGGGATGGTGCTGGCGTCA-3' (SEQ ID NO: 29), and using as template the DNA of clone_λ-1C (Schwecke, T. et al. Proc. Natl. Acad. Sci. USA (1995) 92:7839-7843). Approximately 30-60 ng of the PCR product (4.7 kbp) is digested with uracil DNA glycosylase for 30 minutes at 37°C in the presence of 25 ng pAMP18 vector DNA (Gibco BRL), the mixture is cooled on ice and transformed into E. coli TG1recO and individual colonies are checked for their plasmid content. The desired plasmid (7.4 kbp) is identified by its restriction map and is designated pARRAP.

(Page 49, Line 3) Plasmid pAR32 contains an insert that can be excised by digestion with NdeI and XbaI, but there is an additional NdeI site in the insert that must be specifically protected against cleavage. This is done using the RecA protection method (Koob, M. et al. Nucl. Acids Res. (1992) 20:5831-5835)). The synthetic oligonucleotide 5'-GCACCCACGACGCCACCACCACATATGCCCTGCACCCTGCCCTCC-3' (SEQ ID NO: 30) (in which the NdeI site is underlined) is used together with purified RecA protein and ATP_S, to form a stable triplex DNA-protein complex that specifically protects the internal

NdeI site in rap module 12 from digestion. The protected plasmid pAR32 is digested with NdeI and XbaI, producing the desired full-length insert (13.1 kbp), and this is ligated with plasmid pRM52 (Example 4) which has been digested with NdeI and XbaI. The ligation mixture is transformed into E. coli TG1 recO and individual colonies are screened for their plasmid content. The desired plasmid pAR33 is identified by its restriction pattern.

(Page 50, Line 34) The segment of the ery AI gene from nucleotide 1 to nucleotide 1673, encoding the loading AT-ACP didomain, was amplified by PCR employing the CloneAmp procedure with the following two oligodeoxynucleotides as primers:

5'-ACGCGUACUAGUCCGATTAATTAAGGAGGACCATCAATGGCGGACCTGTCAAAGCTC-3' (SEQ ID NO: 31) and

5'-AUGGAGAUCUCUCCGCTAGCGGTTCGCCGGGCGCCGCTTCGTTGGTCCGCGCGCGGGTTTCCC-3' (SEQ ID NO: 2)

and plasmid pBK6.12 (Example 5) as template, to give plasmid pARLD.

(Page 51, Line 15) The segment of the rapC gene of S. hygroscopicus (Schwecke, T. et al. Proc. Natl. Acad. Sci. USA (1995) 92:7839-7843) from nucleotide 112 to nucleotide 2095, the 5'- end of the DNA encoding rap module 11, is amplified by PCR employing the CloneAmp procedure with the following two oligodeoxynucleotides as primers:

5'-AUGGAGAUCUCUCCGCTAGCGATTGTGGGTATGGCG-3' (SEQ ID NO: 32) and

5'-ACGCGUACUAGUCCATGCATCTGCAGCACGGCGGCCTCATCACCGGA-3' (SEQ ID NO: 33)

and the DNA of recombinant bacteriophage _λ-1C (Schwecke, T. et al., Proc. Natl. Acad. Sci. USA (1995) 92:7839-7843) as the template. Approximately 30-60 ng of the PCR product (2.0 kbp) is digested with uracil DNA glycosylase for 30 min at 37[%]°C in the presence of 25 ng pAMP18 vector DNA, the mixture is cooled on ice and transformed into E. coli TG1 recO and individual colonies checked for their plasmid content. The desired plasmid (4.7 kbp) is identified by its restriction map and is designated pAR11.

(Page 52, Line 1) The segment of the rapC gene of S. hygroscopicus (Schwecke, T. et al., Proc. Natl. Acad. Sci. USA (1995) 92:7839-7843) from nucleotide 7405 to nucleotide 9396, the 3' end of the DNA encoding rap module 12, is amplified by PCR employing the CloneAmp procedure with the following two oligodeoxynucleotides as primers:

5'-ACGCGUACUAGUCCATGCATTCCCGGAGCGGCGATCTGTGG-3' (SEQ ID NO: 34)
and
5'-AUGGAGAUCUCUCCCGCGGCCGCGCTGTCACGCACCAGCTTC AGCAGTGCGTC-3' (SEQ ID NO: 35) and the DNA of recombinant bacteriophage λ-1C (Schwecke, T. et al., Proc. Natl. Acad. Sci. USA (1995) 92:7839-7843) as template. Approximately 30-60 ng of the PCR product (2.0 kbp) is digested with uracil DNA glycosylase for 30 minutes at 37[%]°C in the presence of 25 ng pAMP18 vector DNA, the mixture is cooled on ice and tranformed into E. coli TG1recO and individual colonies are checked for their plasmid content. The desired plasmid (4.7 kbp) is identified by its restriction map and is designated pAR12.

(Page 52, Line 24) The 1.3 kbp segment of the eryAIII gene, extending by 132 nucleotides 3' of the eryAIII stop codon to a KpnI site, and encoding the C-terminal chain-

terminating thioesterase/cyclase of DEBS, is amplified by PCR employing the CloneAmp procedure with the following two oligodeoxynucleotides as primers:
5'-ACGCGUACUAGUCCGCGGCCGCGATCCTCGGGCATTCCAGC-3' (SEQ ID NO: 36)
and
5'-AUGGAGAUCUCUAAGCATTGGTAACTGTC-3' (SEQ ID NO: 37), and plasmid pEXDB3 (Roberts, G. A. et al. Eur J. Biochem. (1993) 214:305-311) as the template. Approximately 30-60 ng of the PCR product (1.3 kbp) is digested with uracil DNA glycosylase for 30 min at 37[%]°C in the presence of 25 ng pAMP18 vector DNA, the mixture is cooled on ice and transformed into E. coli TG1 recO and individual colonies checked for their plasmid content. The desired plasmid (4.0 kbp) is identified by its restriction map and is designated pARTE.

(Page 53, Line 8) The 1.3 kbp segment of plasmid pBR322 containing the tetracycline resistance gene is amplified by the CloneAmp procedure with the following two oligodeoxynucleotides as primers:

5'-ACGCGUACUAGUATCTAGACCATGCATGTTTGACAGCTTATCATC-3' (SEQ ID NO: 38)
and
5'-AUGGAGAUCUCUATCTAGACCATGCATGCCGCCGGCTTCCATTCA-3' (SEQ ID NO: 39)

and plasmid pBR322 as the template. Approximately 30-60 ng of the PCR product (1.3 kbp) is digested with uracil DNA glycosylase for 30 minutes at 37[%]°C in the presence of 25 ng pAMP18 vector DNA, the mixture is cooled on ice and transformed into E. coli TG1recO and individual colonies are checked for their plasmid content. The desired plasmid (4.0 kbp) is identified by its restriction map and is designated pARTr.

(Page 58, Line 15) 50 pmol of each of synthetic oligonucleotides 8985 (5'-GAGCAGTCGTTCCGAGATCTCGGCTTCGATTCA-3'; SEQ ID NO: 40) which introduced a BglII site and 9204 (5'-GGGAGGAGATCAGATCCCAGAAGT-3'; SEQ ID NO: 41) were used by PCR to amplify a 300 bp product from 60 ng pIG70ΔEco. The PCR product was end-repaired, phosphorylated and ligated into pUC18 that had been linearised with SmaI and dephosphorylated. The ligation mixture was used to transform E. coli TG1 recO and individual colonies were checked for their plasmid content. The orientation of pIGPCRstart was identified by a double restriction enzyme digest with EcoRI and BglII to give a pattern that included a 300 bp fragment.

(Page 58, Line 30) 50 pmol of each of synthetic oligonucleotides 8986 (5'-GAGGGAGTCGAACCGAGATCTCGGAACGCGCGG-3'; SEQ ID NO: 42) which introduced a BglII site and 9205 (5'-GGGGGATCCTGGGGTCGGCCGGGCAGGGCAA-3'; SEQ ID NO: 43) were used by PCR to amplify a 440 bp product from 60 ng pIG71ΔSac. The PCR product was end-repaired, phosphorylated and ligated into pUC18 that had been linearised with SmaI and dephosphorylated. The ligation mixture was used to transform E. coli TG1 recO and individual colonies were checked for their plasmid content. The orientation of pIGPCRend was identified by its restriction enzyme digest pattern.

(Page 61, Line 7) For the PCR amplification for plasmid pMO07, the following synthetic oligonucleotides were used as mutagenic primers, one containing a HindIII site and the other an EcoRV site:
5' -GTCTCAAGCTTCGGCATCAGCGGCACCAA- 3' (SEQ ID NO: 44)
and 5' -CGTGCGATATCCCTGCTCGGCGAGCGCA-3' (SEQ ID NO: 45)

(Page 61, Line 13) For the PCR amplification for plasmid pMO08, the following synthetic oligonucleotides

were used as mutagenic primers, one containing a PstI site and the other a HindIII site:
5' -CATGGCCTGCAGGCTGCCCGGGGAGGTCGACT- 3' (SEQ ID NO: 46)
and 5' -CCCGAAGCTTGACACACCTGCCCGGCGCACCCCGT- 3' (SEQ ID NO: 47)

(Page 61, Line 20) For the PCR amplification for plasmid pMO09, the following synthetic oligonucleotides were used as mutagenic primers, one containing a MunI site and the other a PstI site:
5' -GCGCGCCAATTGCGTGCACATCTCGAT- 3' (SEQ ID NO: 48)
and 5' -CCTGCAGGCCATCGCGACGACCGCGACCGGTTCGCCG- 3' (SEQ ID NO: 49)

(Page 63, Line 7) For the PCR amplification for plasmid pKSA, the following synthetic oligonucleotides were used as mutagenic primers, one containing a PstI site and the other a HindIII site:
5' -GATGGCCTGCAGGCTGCCCGGCGGTGTGAGCA- 3' (SEQ ID NO: 50) For the PCR amplification for plasmid pKSA, the following synthetic oligonucleotides were used as mutagenic primers, one containing a PstI site and the other a HindIII site:
5' -GATGGCCTGCAGGCTGCCCGGCGGTGTGAGCA- 3' (SEQ ID NO: 50)
and 5' -GCCGAAGCTTGAGACCCCCGCCCGGCGCGGTCGC- 3' (SEQ ID NO: 51)

(Page 63, Line 14) For the PCR amplification for plasmid pKSB, the following synthetic oligonucleotides were used as mutagenic primers, one containing an EspI site and the other a PstI site:
5' -TGGCTTCGCTGGCGGACACGCTCAG- 3' (SEQ ID NO: 52)
and 5' -CCTGCAGGCCATGCCGACGATCGCGATCGGCT- 3' (SEQ ID NO: 53)

(Page 63, Line 21) For the PCR amplification for plasmid pKSC, the following synthetic oligonucleotides were used as

mutagenic primers, one containing a HindIII site and the other a BspEI site:
5' -GTCAAGCTTCGGGGTGAGCGGGACGAA- 3' (SEQ ID NO: 54)
and 5' -GCGTCCGGACGTGGCTCCAGCA-3' (SEQ ID NO: 55)

(Page 73, Line 6) The approximately 0.46 kbp DNA fragment of the eryAI gene of S. erythraea was amplified by PCR using as primers the synthetic oligonucleotides: 5'-GGAGTACTGCGAGGGCGTGGGCAT-3' (SEQ ID NO: 56) and 5'-CACCTAGGACCGCTTCCCAGTCGACC-3' (SEQ ID NO: 57) and plasmid pNTEPH as template. The PCR product was treated with T4 polynucleotide kinase and then ligated with plasmid pUC18, which had been linearised by digestion with SmaI and then treated with alkaline phosphatase. The ligation mixture was used to transform E. coli DH10B and individual colonies were checked for their plasmid content. The desired plasmid pJLK01 was identified by its restriction pattern and DNA sequencing.

(Page 73, Line 22) The approximately 1.47 kbp DNA fragment of the eryAI gene of S. erythraea was amplified by PCR using as primers the synthetic oligonucleotides: 5'-TACCTAGGCCGGGCCGGACTGGTCGACCTGCCGGGTT-3' (SEQ ID NO: 58) and 5'-ATCCTCAGGCTCTCCGTCTCCGGTTCTCC-3' (SEQ ID NO: 59) and plasmid pNTEPH as template. The PCR product was treated with T4 polynucleotide kinase and then ligated with plasmid pUC18, which had been linearised by digestion with SmaI and then treated with alkaline phosphatase. The ligation mixture was used to transform E. coli DH10B and individual colonies were checked for their plasmid content. The desired plasmid pJLK08 was identified by its restriction pattern and DNA sequencing.

(Page 74, Line 1) The approximately 1.12 kbp DNA fragment of the eryAI gene of S. erythraea was amplified by PCR using as primers the synthetic oligonucleotides:

5'-TACCTGAGGGACCGGCTAGCGGGTCTGCCGCGTG-3' (SEQ ID NO: 60) and 5'-CTTCTAGACTATGAATTCCCTCCGCCCAGC-3' (SEQ ID NO: 61) and plasmid pNTEPH as template. The PCR product was treated with T4 polynucleotide kinase and then ligated with plasmid pUC18, which had been linearised by digestion with SmaI and then treated with alkaline phosphatase. The ligation mixture was used to transform E. coli DH10B and individual colonies were checked for their plasmid content. The desired plasmid pJLK09 was identified by its restriction pattern and DNA sequencing.

(Page 75, Line 22) The approximately 3.3 kbp DNA of the rapC gene of S. hygroscopicus encoding the reduction loop of module 13 was amplified by PCR using as primers the synthetic oligonucleotides: 5'-CGCCTAGGCACCACCACAACCCGGGTACTGGACC-3' (SEQ ID NO: 62) and 5'-TAGCTAGCCGGGCGCTCAGGGGCTGCGAGCCGACCT-3' (SEQ ID NO: 63) and cosmid cos 31 (Schwecke, T. et al. (1995) Proc. Natl. Acad. Sci. USA 92:7839-7843) as template. The PCR product was treated with T4 polynucleotide kinase and then ligated with plasmid pUC18, which had been linearised by digestion with SmaI and then treated with alkaline phosphatase. The ligation mixture was used to transform E. coli DH10B and individual colonies were checked for their plasmid content. The desired plasmid pJLK14 was identified by its restriction pattern and DNA sequencing.

(Page 77, Line 17) The approximately 2.8 kbp DNA fragment of the rapA gene of S. hygroscopicus encoding the reduction loop of module 4 was amplified by PCR using as primers the synthetic oligonucleotides: 5'-CCTAGGCACCACCACGGCCCGGGTGCTGGACCTT -3' (SEQ ID NO: 64) and 5'-CCTCAGGCTGTCACCGGTAGAGGCGGCCCT-3' (SEQ ID NO: 65) and cosmid cos 25 (Schwecke, T. et al. (1995) Proc. Natl. Acad. Sci. USA 92:7839-7843) as template. The PCR product was

treated with T4 polynucleotide kinase and then ligated with plasmid pUC18, which had been linearised by digestion with SmaI and then treated with alkaline phosphatase. The ligation mixture was used to transform E. coli DH10B and individual colonies were checked for their plasmid content. The desired plasmid pJLK16 was identified by its restriction pattern and DNA sequencing.

(Page 79, Line 14) For the PCR amplification of an approximately 1.3 kbp DNA fragment for plasmid pJLK19, the following synthetic oligonucleotides were used as primers:
5' -GTCAAGCTTCGGGGTGAGCGGGACGAA- 3'(SEQ ID NO: 54)
and 5' -ATCCTAGGACCGCTTCCCAGTCGACCGCGACA- 3'(SEQ ID NO: 66) PCR was carried out on pNTEPH as template. The PCR product was treated with T4 polynucleotide kinase and then ligated with plasmid pUC18, which had been linearised by digestion with SmaI and then treated with alkaline phosphatase. The ligation mixture was used to tranform E. coli DH10B and individual colonies were checked for their plasmid content. The desired plasmid pJKL19 was identified by its restriction pattern.

(Page 82, Line 19) The 1.4 kbp segment of plasmid pNTEP2 containing from nucleotide 9838 to 11214 (encoding amino acids 3279 to the end of DEBS1-TE) is amplified by PCR with the following two synthetic oligonucleotides as primers

5'-GCCACTAGTGTGGCGTGGGGGCTGTGGG-3' (SEQ ID NO: 67) and
5'-TGAATTCCCTCCGCCCAGCCAGGCGTCGAT-3' (SEQ ID NO: 68)
and plasmid pNTEP2 as template. The PCR product was end-repaired and ligated with plasmid pUC18, which had been linearised by digestion with SmaI and then treated with alkaline phosphatase. The ligation mixture was

used to transform E. coli DH10B and individual colonies were checked for their plasmid content. The desired plasmid p37 in which an SpeI site was introduced at the 5' end of this fragment was identified by its restriction pattern and by DNA sequencing.

(Page 83, line 10) The 1.1 kbp DNA segment of the eryAI gene of S. erythraea extending from nucleotide 8202 to nucleotide 9306 was amplified by PCR using as primers the synthetic oligonucleotides:
5'-CCTGGAGTACTGCGAGGGCGTG-3' (SEQ ID NO: 69) and
5'-CTGACTAGTGGCGGTGACGTGGGCGGGGGAAA-3' (SEQ ID NO: 70) and plasmid pNTEP2 as template. The PCR product was end-repaired and ligated with plasmid pUC18, which had been linearised by digestion with SmaI and then treated with alkaline phosphatase. The ligation mixture was used to transform E. coli DH10B and individual colonies were checked for their plasmid content. The desired plasmid pSCA7 in which an SpeI site has been introduced at the 3' end of this PCR product was identified by its restriction pattern and by DNA sequencing.

(Page 85, Line 27) The 1.9 kbp segment of pUC1-0 from nucleotide 8715 to 10645 was amplified by PCR using as primers the synthetic oligonucleotides:
5'-CCCCTGCAGCCGGACCGCACCACCCCTCGTGACGA-3' (SEQ ID NO: 71) and
5'-CTTCTAGACTATGAATTCCCTCCGCCCAGC (SEQ ID NO: 61) and the DNA of pUC1-0 as template. The PCR product was end repaired and ligated with plasmid pUC18, which had been linearised by digestion with SmaI and treated with alkaline phosphatase. The ligation mixture was used to transform E. coli DH10B and individual colonies were checked for their plasmid content. The desired plasmid designated p1-0 was identified by restriction analysis and DNA sequencing.

(Page 86, Line 5) The 60bp segment of eryAIII from nucleotide 7006 to 7066 was amplified by PCR using as primers the synthetic oligonucleotides:

5'-GGCGGAACGTCTTCCCGGCGGCACCT-3' (SEQ ID NO: 72) and
5'-CCCCTGCAGCCAGTACCGCTGGGGCTCGAA-3' (SEQ ID NO: 73) and pEXDB3 (Roberts, G. A., et al. (1993) Eur. J. Biochem. 214:305-311) as template. The PCR product was end-repaired and ligated with plasmid pUC18, which had been linearised by digestion with SmaI and then treated with alkaline phosphatase. The ligation mixture was used to transform E. coli DH10B and individual colonies were checked for their plasmid content. The desired plasmid designated pD3P was identified by restriction analysis and DNA sequencing.

(Page 90, Line 18) The approximately 1.3 kbp DNA segment of the eryAI gene of S. erythraea extending from nucleotide 1948 to nucleotide 3273 of eryAI (Donadio, S. et al. Science (1991) 252:675-679) was amplified by PCR employing as primers the synthetic oligonucleotides:
5'-CATGCTCGAGCTCTCCTGGGAAGT-3' (SEQ ID NO: 74) and
5'-CAACCCTGGCCAGGGAAGACGAAGACGG-3' (SEQ ID NO: 75), and plasmid pNTEP2 (Example 5) as template. The PCR product was end-repaired and ligated with plasmid pUC18, which had been linearised by digestion with SmaI and then treated with alkaline phosphatase. The ligation mixture was used to transform E. coli TG1 recO and individual colonies were checked for their plasmid content. The desired plasmid pMO1 (3.9 kbp), in which the StuI site bordering the insert is adjacent to the HindIII site in the polylinker, was identified by its restriction pattern.

(Page 91, Line 1) The approximately 0.85 kbp DNA segment of the rapA gene of S. hygroscopicus, extending from

nucleotide 1643 to nucleotide 2486 of rapA, was amplified by PCR employing as primers the following oligonucleotides:

5'-TTCCCTGGCCAGGGGTCGCAGCGTG-3' (SEQ ID NO: 76) and 5'-CACCTAGGACCGCGGACCACTCGAC-3' (SEQ ID NO: 77), and the DNA from the recombinant bacteriophage _-1E (Schwecke, T. et al. Proc. Natl. Acad. Sci. USA (1995) 92:7839-7843) as the template. The PCR product was end-repaired and ligated with plasmid pUC18, which had been linearised by digestion with SmaI and then treated with alkaline phosphatase. The ligation mixture was used to transform E. coli TG1 recO and individual colonies were checked for their plasmid content. The desired plasmid pMO2 (3.5 kbp) was identified by its restriction pattern.

(Page 91, Line 20) The approximately 1.7 kbp DNA segment of the eryAI gene of S. erythraea extending from nucleotide 4128 to nucleotide 5928 of eryAI, was amplified by PCR employing as primers the synthetic oligonucleotides: 5'-TGGCCAGGGAGTCGGTGCACCTAGGCA-3' (SEQ ID NO: 78) and 5'-GCCGACAGCGAGTCGACGCCGAGTT-3' (SEQ ID NO: 79) and plasmid pNTEP2 as template. The PCR product was end-repaired and ligated with plasmid pUC18, which had been linearised by digestion with SmaI and then treated with alkaline phosphatase. The ligation mixture was used to transform E. coli TG1 recO and individual colonies were checked for their plasmid content. The desired plasmid pMO3 (4.4 kbp), in which the BalI and AvrII sites are adjacent to the HindIII site of the polylinker, was identified by its restriction pattern.

(Page 100, Line 15) The approximately 0.9 kbp DNA segment of the ATX domain was amplified by PCR employing as primers the following oligonucleotides:
5' CTGGCCAGGGCGCGCAATGGCCGAGCAT 3' (SEQ ID NO: 80) and

5' CCCTAGGAGTCGCCGGCAGTCCAGCGCGGCGCCC 3' (SEQ ID NO: 81) using the DNA from the cosmid pSCIN02 as the template. The PCR product was end-repaired and ligated with plasmid pUC18, which had been linearised by digestion with SmaI and then treated with alkaline phosphatase. The ligation mixture was used to transform E. coli TG1 recO and individual colonies were checked for their plasmid content. The desired plasmid pMO33 (3.5 kbp) was identified by its restriction pattern.

(Page 100, Line 30) Plasmid pMO34 is a derivative of pMO6 with a polycloning site inserted after the stop codon of the inserted D1-AT2 gene. Plasmid pMO6 was digested wih EcoRI and HindIII and annealed with two oligonucleotides forming the double-stranded region of the polycloning site:
5' AATTCATAACTAGTAGGAGGTCTGGCCATCTAGA 3'(SEQ ID NO: 82)
and 5' TCGAAGATCTACCGGTCTGGAGGATGATCAATAC 3'(SEQ ID NO: 83).
The mixture was ligated and transformed into E. coli TG1 recO. Individual colonies were checked for their plasmid content. The desired plasmid pMO34 (13.5 kbp) was identified by its restriction pattern.

(Page 104, Line 16) The approximately 1.0 kbp DNA segment of the eryAI gene of S.erythraea extending from nucleotide 6696 to nucleotide 7707 of eryAI (Donadio. S. et al., Science (1991) 252, 675-679) was amplified by PCR employing as primers synthetic oligonucleotides:
5' GGCGGGTCCGGAGGTGTTCACCGAGTT 3'(SEQ ID NO: 84)
and 5' ACCTTGGCCAGGGAAGACGAACACTGA 3' (SEQ ID NO: 85), and plasmid pNTEp2 as a template. The PCR product was end-repaired and ligated with plasmid pUC18, which had been linearised by digestion with SmaI and then treated with alkaline phosphatase. The ligation mixture was used to transform E.coli TG1 recO and individual colonies were checked for their plasmid content. The desired plasmid pMO25 (3.6 kbp), in which the StuI site bordering the insert is adjacent to the HindIII

site in the polylinker, was identified by its restriction pattern.

(Page 105, Line 13) The approximately 0.6 kbp DNA segment of the eryAI gene of S. erythraea extending from nucleotlde 8660 to nucleotide 9258 of eryAI, was amplified by PCR employing as primers the synthetic oligonucleotides:
5' TCCTAGGCCGGGCCGGACTGGTCGACCTGCCGGGTT 3' (SEQ ID NO: 86)
and 5' AAACACCGCGACCTGGTCCTCCGAGC 3' (SEQ ID NO: 87), and plasmid pNTEP2 as template. The PCR product was end-repaired and ligated with plasmid pUC18, which had been linearised by digestion with SmaI and then treated with aIkaline phosphatase. The ligation mixture was used to transform E. coli TG1 recO and individual colonies were checked for their plasmid content. The desired plasmid pMO26 (3.2 kbp), in which the AvrII site is adjacent to the HindIII site of the polylinker, was identified by its restriction pattern.

(Page 112, Line 5) The 250 bp DNA segment of the eryAIII gene of S.erythraea extending from nucleotide 4807 to nucleotide 5052 of eryAIII, was amplified by PCR employing as primers the synthetic oligonucleotides:
5' TTTGCTAGCGATCGTCGGCATGGCGTGCCGGTT3'(SEQ ID NO: 88)
5'CCCACGAGATCTCCAGCATGATCC3' (SEQ ID NO: 89)
The plasmid pEXD3 was used as a template. The PCR product was end-repaired and ligated with pUC18, which had been linearised by digestion with SmaI and then treated with alkaline phosphatase. The ligation mixture was used to transform E.Coli TG1 recO and individual colonies were checked for their plasmid content. The desired plasmid pCAR5 in which the NheI site is adjacent to the EcoRI site of the polylinker, was identified by its restriction pattern and sequence analysis.

(Page 117, Line 1) The 450 bp DNA segment of the eryAI gene of S.erythraea extending from nucleotide 1 to nucleotide 10631 of

eryAI, was amplified by PCR employing as primers the synthetic oligonucleotides:(bases in bold letters denote the restriction enzyme sites).

SphI

5' GGCGGCATGCGGCGGTTCCT3' (SEQ ID NO: 90)

NheI HpaI

5'AAGCTAGCGGTTCGCCGGGCGCCGCTTCGTTGGTCCGCGCGCGGGTTAAC3'(SEQ ID NO: 91)

(Page 117, Line 34) The following complementary synthetic oligonucleotides were synthesised so as when annealed, they would have the necessary pattern at the 5' and 3' ends that is produced by the action of HpaI and NheI respectively

5'AACCCGCGCGCGGACCAACGAAGCGGCGCCCGGCGAACCG3'(SEQ ID NO: 92)

5'CTAGCGGTTCGCCGGGCGCCGCTTCGTTGGTCCGCGCGCGGGTT3'(SEQ ID NO: 93)

At page 136, please insert the following abstract:

ABSTRACT

E 56

A hybrid type I polyketide synthase gene typically containing a starter module and a plurality of heterologous extender modules is used to synthesis novel polyketides. It is preferably under the control of a type II polyketide synthase promoter e.g. act I of S. coelicolor

Marked-Up Copy of Amended Claims

24. (Amended) A hybrid PKS gene according to claim 2, wherein said at least one first nucleic acid portion encodes a loading module together with only the ketosynthase ("KS") domain [(only)] of the [homologous extender] extension module, which is homologous to said loading module.

27. (Amended) A hybrid PKS gene according to claim 26, wherein said loading module is [an avr] the loading module of the avermectin-producing PKS of streptomyces avermitilis.

29. (Amended) A hybrid PKS gene according to claim 1, including a nucleic acid sequence encoding a chain terminating enzyme other than thioesterase.

31. (Amended) [Nucleic acid] A nucleic sequence encoding a gene according to claim 1 operably linked to a PKS type II promoter.

32. (Amended) [Nucleic acid] A nucleic sequence according to claim [11, wherein the promoter is accompanied by its] 31 further comprising the natural activator gene for said promoter.

33. (Amended) [Nucleic acid] A nucleic sequence according to claim 31, wherein the promoter is act I of S. coelicolor.

34. (Amended) [Nucleic acid] A nucleic sequence according to claim 32, wherein the promoter is act I of S. coelicolor.

37. (Amended) A transformed microorganism containing a gene according to claim 1 and able to express a polyketide synthase encoded thereby.

39. (Amended) A method of making a polyketide by culturing the microorganism of claim 37.